REVIEW

The genetic and molecular bases of monogenic disorders affecting proteolytic systems

I Richard

J Med Genet 2005;**42**:529–539. doi: 10.1136/jmg.2004.028118

Complete and limited proteolysis represents key events that regulate many biological processes. At least 5% of the human genome codes for components of proteolytic processes if proteases, inhibitors, and cofactors are taken into account. Accordingly, disruption of proteolysis is involved in numerous pathological conditions. In particular, molecular genetic studies have identified a growing number of monogenic disorders caused by mutations in protease coding genes, highlighting the importance of this class of enzymes in development, organogenesis, immunity, and brain function. This review provides insights into the current knowledge about the molecular genetic causes of these disorders. It should be noted that most are due to loss of function mutations, indicating absolute requirement of proteolytic activities for normal cellular functions. Recent progress in understanding the function of the implicated proteins and the disease pathogenesis is detailed. In addition to providing important clues to the diagnosis, treatment, and pathophysiology of disease, functional characterisation of mutations in proteolytic systems emphasises the pleiotropic functions of proteases in the body homeostasis.

INTRODUCTION

Proteases catalyse the hydrolysis of peptide bonds between two adjacent amino acids. This irreversible reaction is widely used to control biological events both inside and outside the cell. It operates to eliminate damaged proteins, control protein levels, present antigenic peptides, activate precursors, or alter an existing function of a protein. Proteases are therefore key signalling proteins in many processes such as embryonic development, coagulation, immunity, cell differentiation, and cell death.

Proteases are divided into five different groups depending on the type of catalytic reaction they mediate (serine, cysteine, threonine, aspartate, and metallo proteases). They are further subdivided according to the structure of their catalytic sites into clans and families. To date, 504 sequences for proteolytic enzymes are reported for the human genome in the MEROPS database (http://merops.sanger.ac.uk). In addition, 182 non-proteolytic homologues have been identified. Many more genes are involved in proteolysis if the other participants in proteolytic events, such as inhibitors, cofactors or substrates, are taken into account. In particular, it is worth mentioning the very abundant class of E3 ubiquitin ligases, enzymes that determine the specificity of and timing for proteasomal degradation of target proteins.

Mutations in numerous proteases have been implicated in the aetiology of human disorders. Pathogenesis can be caused by uncontrolled activation of proteases resulting from specific gain of function mutations. When a mutation on one allele produces a level of activated proteases sufficient to generate a phenotype, it presents a dominant transmission. Conversely, pathogenesis can be caused by inactivation of the proteolytic activity. The phenotype will then be transmitted in a dominant fashion if the mutation produces a dominant negative form of the protease or in cases of haploinsufficiency. A recessive transmission mode is obtained in case of true loss of function mutations. In this case, half the normal enzyme is enough to fulfil the function, and a phenotype arises only when no activity is present in homozygotes.

The purpose of this review is to provide insights into the current knowledge about the molecular genetic causes of disorders of the proteolytic systems. Recent progress in understanding the function of the implicated proteins and their disease pathogenesis are also presented. This review focuses on monogenic

Correspondence to: Dr I Richard, Généthon CNRS UMR8115, 1, rue de l'internationale, 91000 Evry, France; richard@genethon.fr

Received 12 October 2004
Revised version received 15 November 2004
Accepted for publication 9 December 2004

Abbreviations: ADAMTS, a disintegrin and metalloproteinase with thrombospondin-like motifs; ALPSII, autoimmune lymphoproliferative syndrome type II; APP, amyloid precursor protein; CFI, complement factor I; CLN2, neuronal late infantile ceroid lipofuscinosis; CNS, central nervous system; DISC, death inducing signalling complex; DUB, de-ubiquitinating enzymes; ECE-1, endothelin converting enzyme 1; ECM, extracellular matrix; ENaC, epithelial amiloride sensitive sodium channel; GCSF, granulocyte colony stimulating factor; GCSFR, granulocyte colony stimulating factor receptor; HSCR, Hirschsprung's disease; IKK, IκB kinase; LDLR, low density lipoprotein receptor; LGMD2A, limb girdle muscular dystrophy type 2A; MAD, mandibuloacral dysplasia; MASP2, mannan binding lectin serine protease 2; MMP, matrix metalloproteases; NARC1, neural apoptosis regulated convertase; NE, neutrophil elastase 2; NFκB, nuclear factor kappa B; PCSK9, proconvertase 9; PHEX, *p*hosphate regulating gene with *h*omologies to endopeptidases located on the *X* chromosome; RIP, regulated intramembrane proteolysis Aβ, amyloid β peptides; SPG7, spastic paraplegia; SUMO, small ubiquitin-like modifier; TMPRSS3, transmembrane protease, serine 3; TTP, thrombotic thrombocytopenic purpura; UCH, ubiquitin C terminal hydrolase; USP, ubiquitin specific processing protease; UCHL1, ubiquitin carboxyterminal esterase L1; VWF, von Willebrand factor; XLH, X linked hypophosphataemia

disorders with mutations in genes coding for proteases, describes the pleiotropy and emphasises the importance of the functions of proteases in the body homeostasis.

GENETIC DISORDERS ASSOCIATED WITH SERINE PROTEASES

Serine proteases are a class of abundant proteases in humans. They intervene in proteolytic cascades in the digestive tract and plasma and in various processes occurring in the secretory pathway and at transmembrane level (fig 1A, table 1). The human genetic disorders implicating this class of proteases parallel the diversity of their functions, and can result in problems with digestion, coagulation, complement activation, and impairment in synaptic plasticity, channel activity, prohormone maturation, stem cell differentiation, signalling, and degradation of substrates within the lysosome (fig 1B).

Digestion

The main part of protein digestion is achieved by pancreatic proteases, mainly trypsin (existing as cationic and anionic isoforms) and chymotrypsin. They are synthesised by exocrine cells as the inactive proenzymes trypsinogen and chymotrypsinogen, and are packaged into secretory vesicles together with trypsin inhibitor. Once released into the lumen of the small intestine, trypsinogen is activated to trypsin by enterokinase, a transmembrane enterocyte protease. Trypsin, in turn, activates chymotrypsinogen. Mutations in genes participating to this cascade have been identified in humans.

Gain of function mutations in cationic trypsinogen lead to an autosomal dominant form of chronic pancreatitis,[1 2] which

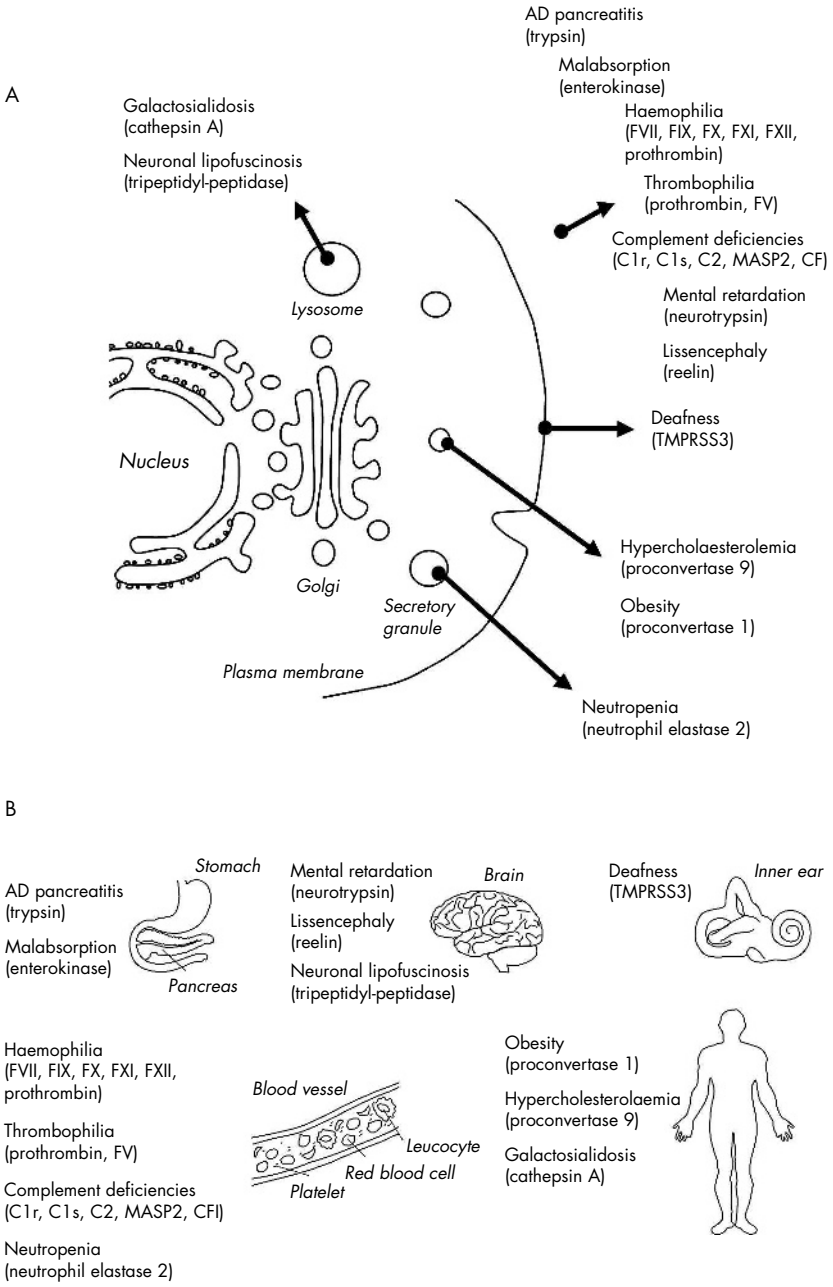

Figure 1 Disorders involving serine proteases. (A) Subcellular localisation of the different proteases involved; (B) the organs presenting the prominent phenotype.

begins with recurrent episodes of acute pancreatitis and progresses to exocrine and endocrine pancreatic insufficiency. These manifestations correspond to an autodigestion of the pancreas due to premature intrapancreatic protease activation of the mutant cationic trypsinogen or to an increased transactivation of anionic trypsinogen.[3 4]

Loss of function mutations in enterokinase cause congenital enteropeptidase deficiency with retardation of growth during childhood, chronic diarrhoea, and generalised oedema.[5] This severe protein malabsorption is prominent during childhood and corresponds to a lack of enterokinase dependent activation of digestive enzymes.

The coagulation cascade

Coagulation is activated in response to a rupture of the vascular epithelium (extrinsic pathway) or by endogenous conditions in the vessels (intrinsic pathway), resulting in platelet aggregation, activation of the coagulation cascade, and finally the formation of fibrin and a clot. The coagulation cascade corresponds to a chain of proteolytic events performed by the procoagulant factors VII, IX, X, XI, and XII, and thrombin, in association with cofactors V and VIII. Several control factors, such as antithrombin, proteins C and S, and plasmin, function as anticoagulants, ensuring that the coagulation is limited to the site of interest. Disturbance in the balance of coagulation can lead either to bleeding diseases or to thrombophilias.

Bleeding disorders present themselves as bleeding episodes in mucosa, joints, muscles, brain, and internal organs. They are mainly due to loss of function mutations in the serine proteases of the coagulation cascade, and include mutations in factor VII, IX (haemophilia B), X, XI (haemophilia C), or XII or in prothrombin.[6 7] With the exception of haemophilia B, which is X linked, they are all transmitted according to autosomal recessive inheritance but with possible manifestations in heterozygotes. In addition, while not affecting a protease per se, there are also loss of function mutations in protease cofactor, such as factor VIII (X linked haemophilia A) and factor V (autosomal recessive).

Thrombophilias are hereditary predispositions to venous thrombosis and can arise by virtue of specific gain of function mutations in procoagulant proteins or loss of function mutations in genes encoding control proteins of the coagulation cascade.[8 9] The gain of function mutations, transmitted in an autosomal dominant manner, have a high prevalence (2–5% in white populations) possibly due to a selective advantage of women during delivery. Only one case corresponds to a serine protease: the G20210A mutation of the 3′ untranslated region of the prothrombin gene, which is associated with a 30% increase in its concentration and activity. The loss of function mutations are rare, with fewer than 1/300–1/2000 individuals affected, and include deficiencies of the serine proteases, protein C, tissue plasminogen activator, and plasminogen.

Complement activation

Complement is a major part of the innate immune system, and also participates in the adaptive immune response.[10 11] Complement can be activated according to three different pathways: classic, mannose binding lectin, or alternative. These pathways correspond to a series of proteolytic events, sequentially activating proteins of the complement and ultimately producing a key enzyme called C3 convertase. A common final pathway follows the generation of C3 convertase, leading to the formation of the membrane attack complex that destroys microorganisms. Several genetic conditions involving the serine proteases of the cascades have been identified, all of which correspond to loss of function mutations, but with various immune consequences.

In the classic pathway, deficiencies in the serine proteases C1r, C1s and C2 have been described to lead to multiple autoimmune features, especially systemic lupus erythematosus, an autoimmune manifestation affecting skin, joints, and other organs.[12–15] To reconcile the deficiencies in activator proteases with what appears to be a hyperactivation of the complement, the ''waste disposal'' hypothesis has been proposed.[11] In this hypothesis, the complement system fails to eliminate cells that have undergone apoptosis, and the partially degraded components of those cells induce an autoimmune response.

In the lectin pathway, one case of mannan binding lectin serine protease 2 (MASP2) deficiency has been reported.[16] MASP2 is recruited to form the enzymatically active mannan binding lectin–MASP complex, and thus generating C3 convertase. The homozygous missense mutation of this patient prevents MASP2 binding to lectin and leads to complement deficiency. The disorder manifests itself as a susceptibility to infections and chronic inflammatory signs.

In the alternative pathway, inherited recessive deficiency of complement factor I (CFI), a C3 inactivating protease, have been described in two families.[17] The deficiency causes an uncontrolled activation of the alternative pathway resulting in secondary C3 depletion. Consequently, patients with CFI deficiency present a propensity to acquire opportunist infections.

Disorders involving miscellaneous serine proteases

Loss of function mutations in neurotrypsin, a brain serine protease, lead to a rare non-syndromic learning disabilities with autosomic recessive transmission.[18] Patients have cognitive impairment with IQ <50. Neurotrypsin is secreted through presynaptic vesicles by neurones of brain areas important in learning and memory.[19] Loss of function mutations seem to hamper the structural reorganisations accompanying the synaptic plasticity in these processes.

Loss of function mutations in the transmembrane protease, serine 3 (TMPRSS3), a protease of epithelia such as cochleae, have been associated with congenital or childhood onset deafness with autosomal recessive inheritance (DFNB10 and DFNB8).[20] It has been shown that the epithelial amiloride sensitive sodium channel (ENaC) is activated by autocatalytically processed TMPRSS3.[21] ENaC function is critical for normal salt and water regulation, one of its functions being to maintain the low concentration of Na in the endolymph of the inner ear.[22] This electrochemical composition is necessary for the transformation of sound into nerve impulses by sensory hair cells. Therefore, the pathophysiological cascade seems to be defective proteolytic activity of TMPRSS3, impairment of EnaC activation, and endolymph sodium imbalance, resulting in hearing loss.

Mutations in proprotein convertases, a group of proteins responsible for the maturation of proteins and peptides within the secretory pathway, have been identified in two human conditions. A woman with a syndrome of obesity and multiendocrine disturbances was found to carry two loss of function mutations in the prohormone convertase-1.[23] This enzyme is strongly expressed in neuroendocrine cells and is responsible for maturation of prohormones such as proinsulin and pro-opiomelanocortin. Their impaired processing leads to abnormalities of glucose homeostasis and adrenal function.[24] Missense mutations in proconvertase 9 (*PCSK9*) have been found in families with autosomal dominant hypercholesterolaemia.[25 26] *PCSK9* encodes neural apoptosis regulated convertase (NARC1), strongly expressed in liver and small intestine.[27] Overexpression of this protease decreases the level of the low density lipoprotein receptor (LDLR), which is responsible for clearing LDL from plasma.[28] It is therefore tempting to speculate that mutations in NARC1

Table 1 Serine proteases and their genetic disorders

Gene	Protein	Disorder	Trans	Function	MIM
PRSS1	Cationic trypsin	Pancreatitis	AD	Digestion	167800
PRSS7	Enterokinase	Malabsorption	AR	Digestion	226200
F7	Factor VII	Haemophilia	AR	Procoagulation	227500
F9	Factor IX	Haemophilia B	X linked	Procoagulation	306900
F10	Factor X	Haemophilia	AR	Procoagulation	227600
F11	Factor XI	Haemophilia C	AR	Procoagulation	264900
F12	Factor XII	Haemophilia	AR	Procoagulation	234000
F2	Prothrombin	Haemophilia	AR	Procoagulation	176930
F2	Prothrombin	Thrombophilia	AD	Procoagulation	176930.0009
PROC	Protein C	Thrombophilia	AR	Anticoagulant	176860
PLAT	Tissue plasminogen activator	Thrombophilia	AR	Anticoagulant	173370
PLG	Plasminogen.	Thrombophilia	AR	Anticoagulant	173350
C1R	C1r	Complement deficiency	AR	Complement	216950
C1S	C1s	Complement deficiency	AR	Complement	120580
C2	C2	Complement deficiency	AR	Complement	217000
MASP2	MASP2 protein	Complement deficiency	AR	Complement	605102
CFI	Complement factor I	Complement deficiency	AR	Complement	217030
PRSS12	Neurotrypsin	Learning disabilities	AR	Remodelling of nervous tissue	249500
TMPRSS3	Transmembrane protease, serine 3	Deafness	AR	Activation of ENaC	605316 DFNB10, 601072 DFNB8
PC1	Prohormone convertase-1	Obesity	AR	Processing of prohormone	600955
PCSK9	NARC1	Hyper-cholesterolaemia	AD	Cholesterol level	603776
ELA2	Neutrophil elastase	Neutropenia	AD	Differentiation myeloid precursors	202700, 162800
RELN	Reelin	Lissencephaly	AR	Not known	257320
CLN2	Tripeptidyl-peptidase I	Ceroid lipofuscinosis	AR	Lysosome	204500
CTSA	Cathepsin A	Galactosialidosis	AR	Lysosome	256540

Trans, mode of transmission; AD, autosomal dominant; AR, autosomal recessive.

may lead to an excess of proteolytic cleavage of LDLR, preventing LDLR mediated LDL cholesterol uptake.

Neutrophil elastase 2 (NE, encoded by the *ELA2* gene) mutations have been identified as the cause of cyclic and congenital neutropenias, autosomal dominant disorders characterised by a low level of peripheral blood neutrophils and recurring severe bacterial and fungal infections.[29 30] In cyclic neutropenia, the circulating neutrophil number oscillates from normal to extremely low levels with a 21 day periodicity. In both conditions, neutropenia is associated with a marked elevation in the number of monocytes. As neutrophils and monocytes derived from the same myeloid progenitors, these observations are suggestive of an aberrant switch in cell fate. Normally, NE, expressed in bone marrow progenitors of the granulocytic lineage, is processed through the Golgi apparatus, and the mature NE is stored in azurophil granules until released at sites of inflammation. Its implication in neutropenia suggests that it plays an unexpected role in the regulation of haematopoietic stem cell differentiation.[31] Disruption of ELA2 perturbs the intracellular trafficking of NE, suggesting the possibility of defective interactions between mutant NE and membrane proteins.[32 33] Several studies suggest that these could be proteins of the Notch family, as mutant NE affects Notch signalling, a pathway implicated in the differentiation of hematopoetic stem cells.[34] Other candidates are granulocyte colony stimulating factor (GCSF), a growth factor used in treatment of neutropenia, and its receptor (GCSFR), as both are substrates of NE.[35]

Mutations in reelin, an extracellular matrix protein playing a pivotal role in neuronal migration during development, are responsible for lissencephaly, a congenital malformation of the central nervous system (CNS).[36] Reelin binds to the very low density lipoprotein receptor and to the APOE receptor 2 at the cell surface, inducing a signalling cascade to regulate the CNS layer formation.[37] It has also been demonstrated that reelin is a serine protease, of which substrates are extracellular proteins such as laminin and fibronectin.[38] The proteolytic activity of reelin on adhesion molecules of the extracellular matrix and/or neurone receptors may explain how reelin regulates neuronal migration and synaptic plasticity. However, it is not yet known whether the pathogenesis of lissencephaly is dependent or not on its protease activity.[38]

Loss of function mutations in serine proteases of the lysosome lead to two autosomal recessive forms within a group of more than 40 heritable disorders caused by deficiency of lysosomal enzymes. These disorders, grouped under the generic term of lysosomal storage diseases, are characterised by the progressive accumulation of non-metabolised substrates within the lysosome, leading to cellular and tissue damage, organ dysfunction, and early mortality. Deficiency in tripeptidylpeptidase I causes neuronal late infantile ceroid lipofuscinosis (CLN2), a neurodegenerative disorder characterised by seizures, myoclonus, learning disabilities, and ataxia.[39] Mutations in lysosomal carboxypeptidase or cathepsin A are responsible for late infantile and juvenile galactosialidosis, presenting with variable clinical features including skin and neuronal involvements.[40] The resulting complete loss of cathepsin A enzymatic activity leads to a secondary deficiency in neuraminidase and β-galastosidase. These three enzymes are associated in a large multienzymatic complex in which cathepsin A acts as a protective protein towards the two other enzymes, preventing their degradation.[41]

GENETIC DISORDERS ASSOCIATED WITH METALLOPROTEASES

Only seven genetic conditions have been associated with mutations in metalloproteases (table 2), even though the metalloproteases are more numerous than serine proteases in the human proteome. Nevertheless, the clinical manifestations and underlying pathological mechanisms are also very diverse. Extracellular matrix remodelling, peptide and protein processing in extracellular or intracellular compartments, and quality control of mitochondrial proteins are altered in these inherited disorders, which affect diverse zinc metalloprotease families (fig 2). Besides these genetic disorders, metalloproteases, especially matrix metalloproteases (MMP), have long been linked with cancer progression. Their activation is increased in almost all human cancers and they have been shown to contribute to tumour invasion through extracellular matrix (ECM) degradation and to tumour angiogenesis.[42] MMP have also been associated with neuroinflammation, multiple sclerosis, and rheumatoid arthritis.[43 44]

Disorders involving matrix metalloproteases

Loss of function mutations in MMP-2 (also known as gelatinase A) have been found in a Saudi family affected by autosomal recessive multicentric osteolysis with arthritis.[45] Clinical manifestations include dysmorphic facial features, short stature, marked carpal and tarsal ostepenia, and distal arthropathy progressing towards ankylosis. MMP-2 is expressed in osteoblasts, osteoclasts, and fibroblasts, and was shown to cleave several ECM proteins such as type IV collagen, elastin, fibronectin, and laminin.[46] It is postulated that incomplete degradation of the ECM caused by the lack of MMP2 activity could circumvent an appropriate osteoblast bone deposition.

Disorders involving a disintegrin and metalloproteinase with thrombospondin-like motifs (ADAMTS)

Loss of function mutations in ADAMTS2 are responsible for Ehlers-Danlos syndrome type VIIC.[47] This autosomal recessive congenital condition is mainly characterised by characteristic facies, extreme skin fragility, and joint laxity. ADAMTS2 is a procollagen N proteinase involved in procollagen processing.[48] Collagen maturation involves the cleavage of N and C terminal propeptides to produce mature monomers capable of forming fibrils. The deficiency in ADAMTS2 results in defect in the N terminal processing of procollagen leading to unprocessed precursors. These, once assembled into abnormal ''hieroglyphic'' collagen fibrils, do not provide the normal tensile strength to skin tissue.

Loss of function mutations in ADAMTS13 lead to thrombotic thrombocytopenic purpura (TTP),[49] characterised by intravascular destruction of red blood cell and platelets. ADAMTS13 was identified as the cleaving protease of von Willebrand factor (VWF), the platelet adhesive blood coagulation protein.[49] VWF is secreted by vascular endothelial cells and released into plasma as large multimers exhibiting a high affinity for platelets and collagen.[50] In normal human plasma, VWF is rapidly cleaved into smaller forms by ADAMTS13. In TTP, the absence of VWF multimer processing results in enhanced platelet aggregation, eventually leading to microvascular thrombosis and haemolysis.

Disorders involving metalloproteases of the neprilysin family

Neprilysin family members are type II transmembrane proteases involved in the extracellular metabolism of biological active peptides.[51] Their main role lies in the control of intercellular communication through activation or inactivation of peptidic signals.

A mutation in endothelin converting enzyme 1 (ECE-1) was reported in one patient with a form of Hirschsprung's disease (HSCR) associated with cardiac defects and craniofacial abnormalities.[52] HSCR is a congenital disorder characterised by a total or partial absence of the nerve cells innervating the intestine. The resulting lack of peristalsis prevents intestinal bolus transit, leading to functional obstruction and megacolon. ECE-1 is involved in the proteolytic maturation of endothelins 1 and 3 (ET1 and ET3), two potent vasoconstrictor peptides important for the development of neural crest derived cells from which the enteric nervous system arises.[53 54] The mutation induces a decrease in ECE-1 activities and is therefore expected to reduce the level of mature ET.[52] This may result in disturbance of ET mediated intercellular signalling, which is necessary for migration, proliferation, or differentiation of neural crest cells to form enteric nervous ganglia.[55]

Mutations in PHEX (*p*hosphate regulating gene with *h*omologies to *e*ndopeptidases located on the *X* chromosome) endopeptidase cause X linked hypophosphataemia (XLH).[56] This disorder is characterised by a renal tubular abnormality resulting in phosphate wasting and defective bone mineralisation, leading to growth retardation and progressive severe skeletal abnormalities. Several studies indicated that a phosphaturic hormone, phosphatonin, may play an important role in the pathophysiological cascade responsible for XLH.[57] The inactivating mutations present throughout the entire length of PHEX lead to a failure of phosphatonin clearance from the circulation by abolishing its degradation. Phosphatonin interacts with a renal tubule cell receptor that in turn downregulates a sodium dependent phosphate cotransporter, resulting in an excessive urinary phosphate excretion. Phosphatonin is also thought to be the mineralisation inhibitor shown to accumulate in mutant osteoblasts and responsible for the inhibition of extracellular matrix mineralisation.[58]

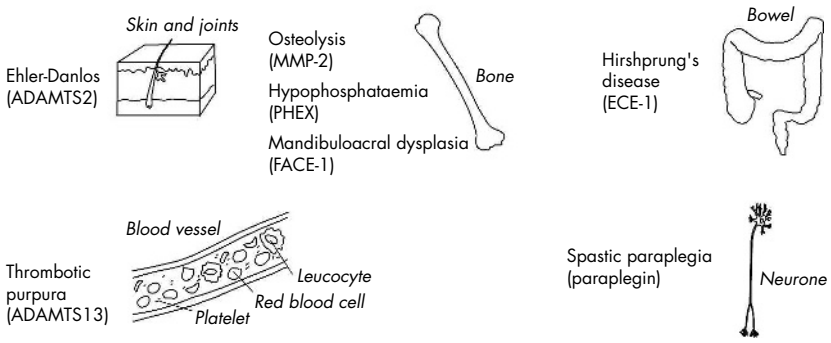

Figure 2 Disorders involving metalloproteases with the organ presenting the prominent phenotype.

Table 2 Metalloproteases and their genetic disorders

Gene	Protein	Disorder	Trans	Function	MIM
ADAMTS2	Procollagen N proteinase	Ehlers-Danlos syndrome	AR	Collagen maturation	225410
ADAMTS13	von Willebrand factor cleaving protease	TTP	AR	VWF cleavage	274150
MMP2	Matrix metalloprotease type 2	Osteolysis	AR	ECM remodelling	605156
ECE-1	Endothelin converting enzyme	Hirschprung's disease	AR	Neural cell crest development	142623
PEK	PHEX	Hypo-phosphataemia	X linked	Clearance of phosphatonin	307800
ZMPSTE24 X	FACE1	Mandibuloacral dysplasia	AR	Prelamin A maturation	248370
SPG7	Paraplegin	Spastic paraplegia	AR	Quality control of mitochondrial proteins	607259

Trans, mode of transmission; VWF, von Willebrand's disease; ECM, extracellular matrix;

Disorders involving other metalloproteases

Mutations in FACE-1 (or ZMPSTE24), a multispanning membrane zinc metalloproteinase, were found in one patient with mandibuloacral dysplasia (MAD).[59] MAD is an autosomal recessive disorder characterised by skeletal abnormalities including hypoplasia of the mandible and clavicles, acro-osteolysis, progeroid appearance, and generalised lipodystrophy. FACE-1 is localised to the ER and the nuclear envelope and is involved in maturation of lamin A, an intermediate filament protein of the nuclear lamina.[60] Processing of prelamin A involves a complex series of post-translational modifications of the C terminus (farnesylation, proteolytic cleavage, and methylation), increasing its hydrophobicity and subsequently facilitating its association with the nuclear lamina. FACE-1 mutations affect prelamin A proteolysis and may compromise its function in nuclear structure, chromatin organisation, or gene regulation.

Mutations in paraplegin, a nuclear encoded mitochondrial metalloprotease cause an autosomal recessive form of hereditary spastic paraplegia (SPG7).[61] SPG7 is characterised by progressive weakness and spasticity of the lower limbs due to selective retrograde degeneration of corticospinal axons. Paraplegin is homologous to yeast mAAA (*A*TPases *a*ssociated with a variety of cellular *a*ctivities) proteases, which associate in a multimeric complex at the mitochondrial inner membrane. This complex exhibits both a chaperone-like activity controlling the assembly of respiratory complexes and a protease activity necessary for the removal of misfolded respiratory chain subunits.[62] SPG7 biopsies showed an accumulation of mitochondriae, signifying a defect in oxidative phosphorylation.[61] A defective respiration seems to be particularly deleterious to long axons, possibly by affecting the energy demanding anterograde and retrograde axonal transport.[63]

GENETIC DISORDERS ASSOCIATED WITH ASPARTYL PROTEASES

Only one genetic condition is associated with aspartyl proteases and corresponds to the autosomal dominant early onset forms of Alzheimer's disease caused by mutations in presenilins 1 (PS1) and 2 (PS2) (table 3).

Table 3 Aspartyl proteases and their genetic disorders

Gene	Protein	Disorder	Trans	Function	MIM
PRS1	Presenilin 1	EOAD	AD	Aβ generation	AD3 607822
PRS2	Presenilin 2	EOAD	AD	Aβ generation	AD4 600759

EOAD, early onset Alzheimer's disease; AD, autosomal dominant; AR, autosomal recessive.; Trans, mode of transmission.

Alzheimer's disease is characterised by dementia associated with neurone loss, extracellular accumulation of amyloid plaques, and intracellular neurofibrillary tangles of the microtubule associated protein tau. It is the most frequent neurodegenerative disorder, affecting 1% of the population over 65 years of age. Most of the forms are sporadic and age of onset is in the seventies (late onset Alzheimer's disease). The familial forms are rare and occur before the age of 60 years (early onset Alzheimer's disease). Mutations in PS1 account for half of the familial cases, and mutations in PS2 for a few percent. Presenilins are heterodimeric membrane proteins generated by endoproteolysis of precursor proteins.[64] They have been shown to correspond to the proteolytic part of γ-secretase, a macromolecular complex involved in intramembrane proteolysis of several transmembrane proteins including amyloid precursor protein (APP) and Notch.[65 66] This processing particularly occurs within the lipidic environment of the membranes and is known as regulated intramembrane proteolysis (RIP).[67] More than 100 mutations have been described in PS1, and eight mutations in PS2 (in both cases, most were missense mutations) have been described in PS1 and PS2, respectively. All result in an accumulation of APP proteolytic fragments, the amyloid-β peptides (Aβ), especially the insoluble neurotoxic Aβ42 form, in the brain.[68] Despite intense research, the mechanism by which PS mutations result in increased production of Aβ42 is not fully elucidated, although it is suggestive of an alteration of the selectivity of γ-secretase mediated cleavage.

GENETIC DISORDERS ASSOCIATED WITH CYSTEINE PROTEASES

Cysteine proteases are another class of abundant proteases and include the cathepsins, calpains, caspases, deubiquitinating enzymes, and small ubiquitin-like modifier (SUMO) proteases. Two human monogenic conditions have been associated with defects in cathepsin genes, two with caspases, one with a calpain, three for the deubiquitinating enzymes, but none for the SUMO proteases (fig 3, table 4).

Disorders involving cysteine cathepsins

In addition to their well known role in the non-specific degradation of proteins in the lysosomes, the cysteine cathepsins also participate in specialised functions such as prohormone processing, antigen presentation, bone remodelling, spermatogenesis, angiogenesis, apoptosis, and homeostasis of the skin and hair follicles.[69] A recent report provided evidence that they may even play a role within the nucleus, as a cathepsin L isoform was found to proteolytically activate a transcription factor.[70] Increased

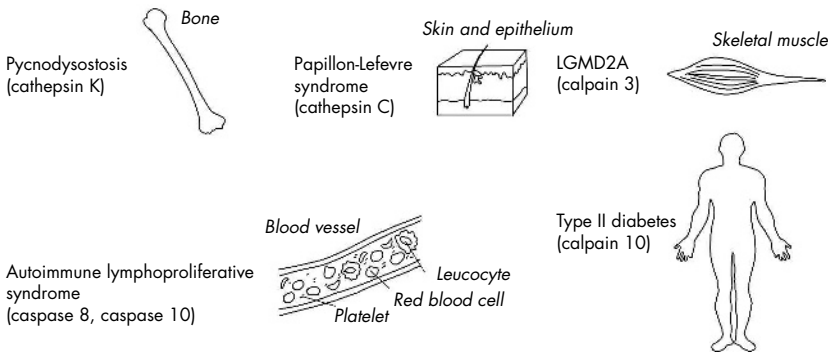

Figure 3 Disorders involving cysteine proteases with the organ presenting the prominent phenotype.

activity of cathepsins, especially cathepsin B, has been associated with tumour growth, invasion, and metastasis in a number of cancers.[69] Upregulation was also observed in degenerative joint disorders, inflammatory myopathies, and artherosclerosis.[69] In contrast, the two human genetic pathologies associated with cathepsins correspond to an inactivation.

Loss of function mutations in cathepsin K cause pycnodysostosis, a rare autosomal recessive osteochondrodysplasia.[71] Patients exhibit bone abnormalities such as a short stature, predisposition to bone fractures, cranial deformation, abnormalities in dentition, aplasia of the terminal phalanges, and abnormal density of the entire skeleton. Cathepsin K is strongly and selectively expressed in osteoclasts, specialised cells of the bone that demineralise and digest the bone matrix during the continuous process of bone remodelling. Cathepsin K deficient osteoclasts are no longer capable of degrading the collagen fibres, but demineralisation of bone still occurs. Fibroblast mediated degradation of collagen of soft connective tissues is also affected.[72]

Inactivating mutations in the cathepsin C (dipeptidyl-peptidase I) gene result in Papillon-Lefevre or in Haim-Munk syndromes, two rare autosomal recessive disorders.[73 74] These allelic disorders are characterised by palmoplantar hyperkeratodermy and early onset periodontal destruction leading to tooth loss. Cathepsin C is expressed in skin and gingival epithelia and in osteoclasts and has been implicated in a variety of immune and inflammatory processes. It is implicated in the activation of several lymphocyte and neutrophil serine proteases which participates in cytotoxic apoptosis and in the regulation of cytokine production.[75] The proposed pathophysiological mechanisms leading to the phenotype include a reduced host response against pathogens in the oral cavity, a loss of alveolar bone, an abnormal differentiation affecting the junctional epithelium that normally binds the ginviga to the tooth surface, or a combination of all of those.

Disorders involving calpains

The calpain family comprises intracellular calcium dependent cysteine proteases.[76] The ubiquitously expressed μ and m calpains have been the most widely studied calpains, whereas information on function and regulation of most other members of the calpain family is scarce. Overactivation of ubiquitous calpains following disturbance in calcium homeostasis has been observed in many pathological conditions including heart ischaemia and acute (stroke and trauma) and degenerative neurological disorders (Alzheimer's and Huntington's diseases).[76 77] Calpain 10 appears to be a susceptibility gene for type 2 diabetes in some populations and calpain 9 seems to be a gastric cancer suppressor.[78 79] However, the only case of monogenic disorder associated with a calpain is recessive limb girdle muscular dystrophy type 2A (LGMD2A) caused by loss of function mutations in the *calpain 3* gene.[80]

LGMD2A is characterised by progressive atrophy of the proximal limb muscles, especially those of the posterior compartment, elevated serum creatine kinase, and a necrosis regeneration pattern on muscle biopsies.[81] Calpain 3 is mainly expressed in skeletal muscle, where it can be found either in the nucleus or the cytoplasm, associated with titin, a giant protein of the sarcomere. Among its substrates are the cytoskeletal proteins, filamin C, talin, vinexin, ezrin, and titin.[82] Calpain 3 deficiency alters the survival pathway of NFκB, leading to an increased level of apoptotic myonuclei.[83] It has been proposed that cleavage of the cytoskeletal proteins is part of an adaptive response to mechanical constraints supported by the muscle. Failure to adapt would

Table 4 Cysteine proteases and their genetic disorders

Gene	Protein	Disorder	Transmission	Function	MIM
CTSK	Cathepsin K	Picnodystosis	AR	Bone remodelling	265800
CTSC	Cathepsin C	Papillon-Lefevre syndrome	AR	Processing of serine proteases	245000
CASP10	Caspase 10	ALPSII	AD and AR	Immune cell apoptosis	603909
CASP8	Caspase 8	ALPSII	AR	Immune cell homeostasis	607271
CAPN3	Calpain 3	LGMD2A	AR	Cytoskeleton remodelling	253600
UCHL1	Ub C terminal esterase 1	Parkinson's disease	AD	Degradation of α-synuclein	168600
CYLD1	Cyld protein	Cylindromatosis	AD	Negative regulator of the NFκB pathway	132700
USP9Y	Ubiquitin specific protease 9, Y chromosome	Azoospermy	Y linked	Spermatogenesis	415000

AD, autosomal dominant; AR, autosomal recessive.

lead to increased cell death, explaining the atrophic phenotype.

Disorders involving caspases

Caspases are known as the proteases of apoptosis, a genetically programmed form of cell death essential for embryonic development, immunity and the pathology of many disorders.[84] Apoptotic pathways can be triggered by a number of pathological and physiological stimuli in an extrinsic or intrinsic manner. In the extrinsic pathway, the origin of the death stimulus is external to the cell and involves assembly of an apical death inducing signalling complex (DISC).[85] In the intrinsic pathway, the stimulus arises from inside a damaged cell and involves mitochondrial permeabilisation.[85] Initial stimuli are followed by a cascade of proteolytic events leading eventually to destruction of architectural components of the cell and DNA fragmentation. Caspases are classified as initiators or executioners depending on their place in the apoptotic cascade. The initiator caspases of the extrinsic pathway are caspases 8 and 10, and that of the intrinsic pathway, caspase 9. An inappropriate level of apoptosis is known to contribute to the pathogenesis of several proliferative or degenerative disorders.[86] Excessive apoptosis occurs in traumatic or ischaemic tissue injury, acquired immunodeficiency syndrome, type I diabetes mellitus and degenerative neurological disorders such as amyotrophic lateral sclerosis, and Alzheimer's, Huntington's, and Parkinson's diseases. Insufficient apoptosis participates in autoimmunity and malignant processes. In particular, somatic mutations in caspases 3, 7, 8, and 10 have been observed in cancer.

Besides these dysregulations, the triggering of which is not well understood in many cases, germline mutations in the two initiator caspases of the extrinsic cell death pathway have been observed in autoimmune lymphoproliferative syndrome type II (ALPSII). ALPSII is characterised by an aberrant proliferation of lymphocytes, resulting in adenopathies and splenomegaly, and is associated with autoimmune and inflammatory manifestations. Two caspase 10 mutations (one recessive, one dominant) have been found in ALPSII families,[87] and the study showed that the dominant mutation acts in a dominant negative manner, probably at the DISC level, to impair the death receptor induced apoptosis in lymphocytes and dendritic cells, whereas the recessive mutation has a less severe apoptotic defect. Homozygous individuals for a caspase 8 mutation, presenting ALPS symptoms together with immunodeficiency, have been identified in one family.[88] In addition to inducing defective lymphocyte apoptosis by a decrease in caspase 8 recruitment to the DISC, the mutation impairs the activation of T and B lymphocytes and of natural killer cells, as demonstrated by the absence of activation markers at the cell surface and defects in cytokine and immunoglobulin production.

Disorders involving deubiquitinating enzymes

Ubiquitination is a reversible post-translational modification of proteins, which controls many cellular functions. An ubiquitin chain consisting of at least four ubiquitins constitutes a signal of degradation by the proteasome.[89] Other modifications are implicated in regulatory mechanisms such as control of receptor internalisation, intracellular trafficking, DNA repair, or transcriptional activity. Deubiquitinating enzymes (DUB) are cysteine proteases of the ubiquitin pathway that specifically cleave ubiquitin from ubiquitin precursors and ubiquitin protein conjugates, ensuring correct equilibrium of these processes with ubiquitin recycling.[90] To date, the DUBs have been divided into the ubiquitin C terminal hydrolase (UCH) and the ubiquitin specific processing protease (UBP or USP) families, on the basis of conserved sequence motifs.[90] In the human genome, four and 63 distinctive genes encoding UCH and UBP respectively, have been identified. An ever increasing number of mutations are being found in DUB.

A mutation in ubiquitin carboxyterminal esterase L1 (UCHL1) was identified in a family with a dominant form of Parkinson's disease,[91] characterised by a selective loss of dopaminergic neurones and the presence of Lewy bodies, inclusions mainly composed of α-synuclein, a presynaptic protein implicated in neuronal plasticity or vesicle transport.[92] In addition to its hydrolase activity, UCHL1 has also been shown to possess ubiquitin ligase activity.[93] The mutated UCHL1 has a reduced catalytic activity that may affect the degradation of α-synuclein leading to its accumulation and aggregation.[91] Interestingly, a polymorphic variant of UCHL1 with reduced ligase activity but comparable hydrolase activity with the wildtype enzyme has been associated with a decreased susceptibility for Parkinson's disease.[94]

Truncating mutations in the tumor suppressor CYLD1 cause cylindromatosis, an autosomal dominant predisposition to benign skin tumours.[95 96] CYLD1 has sequence homology to UCH and has been shown to negatively regulate the NFκB pathway through deubiquitination of TNFα receptor associated factors (TRAF), major mediators of the TNF signalling.[97–99] CYLD dysfunction results in excessive ubiquitination of TRAF, an activation signal in this pathway.[100] The consequences are activation of IκB kinase (IKK), subsequent degradation of IκBα, the inhibitor of NFκB, and ultimately excessive NFκB activation. This activation triggers cell transformation through increased resistance to apoptosis.[97]

Loss of function mutations in ubiquitin specific protease 9 (USP9Y; also named DFFRY) is associated with azoospermy, the absence of sperm production.[101] USP9Y is a Y chromosome gene that possesses a homologue on the X chromosome (DFFRX), both presenting homology with *Drosophila* developmental gene fat facets (faf),[102] important for the normal development of embryo and eye, preventing the proteasomal degradation of specific proteins.[103] By similarity, efficient progression of spermatogenesis might require the stabilisation of particular proteins.

GENETIC DISORDERS OF OTHER COMPONENTS OF PROTEOLYTIC SYSTEMS

This review focuses on proteases. Nevertheless, it is worth mentioning briefly instances in which mutations in other components of proteolytic systems contribute directly to disease pathogenesis. For example, several human genetic disorders have been associated with mutations in protease inhibitors such as serpins, cystatins, and tissue inhibitors of metalloproteinases, which are inhibitors of serine, cysteine and metalloproteases, respectively.[104–108] Defective proteasome dependent proteolysis is also encountered in cases of inefficient substrate recognition caused by mutations in E3 ubiquitin ligases or in substrates themselves, often leading to a toxic accumulation of substrates within the cells. E3 ubiquitin ligases recognise and catalyse the ubiquitin conjugaison to specific substrates for degradation by the proteasome or modulation of protein activities.[109] Five human disorders have been associated to date with mutations in such enzymes: limb girdle muscular dystrophy type 2H, Angelman syndrome, Lafora disease, autoimmune polyendocrinopathy candidiasis ectodermal dystrophy, a form of Fanconi anaemia, and an autosomal recessive form of juvenile Parkinson's disease.[110–120] It is interesting to note that most of the disorders associated by E3 ubiquitin ligases are transmitted in a recessive manner, whereas those associated with DUB are transmitted in a dominant fashion. Alterations of the structure of a substrate can also modify its degradation by the ubiquitin/proteasome system. In

particular, mutation can result in misfolding of the protein, rendering it susceptible to aggregation and preventing it being efficiently recognised and degraded by the ubiquitin proteasome machinery. This mechanism underlies the pathogenesis of several major human neurodegenerative disorders: a dominant form of Parkinson's disease with accumulation of α-synuclein, Alzheimer's disease with accumulation of amyloid precursor protein, and Huntington's disease and spinocerebellar ataxias associated with polyglutamine expansions.[121] Furthermore, there is supporting evidence that the protein aggregrates can compromise the ubiquitin/proteasome function by secondary sequestration of its components.[122]

CONCLUSION AND PERSPECTIVES

To date, 42 inherited disorders have been identified in a total of approximately 500 proteases. As this accounts for less than 10%, it is likely that they represent only a fraction of all existing disorders. It should be noted that the majority of these disorders are of recessive inheritance with loss of function mutations, indicating absolute requirement of these proteolytic activities for normal cellular function. All the examples presented in this review illustrate the role of proteolysis in processes as diverse as digestion, coagulation, regulation of the immune system, regulation of channel activity, peptide and hormone metabolism, gene regulation, clearance and quality control of proteins and tissue development, differentiation, and plasticity. This impressive list highlights the importance for a cell to have tools at its disposal performing irreversible modifications in order to orientate signalling.

Identification of a causative gene helps to understand its physiological function by providing a unique opportunity to examine the consequences of the perturbation of a specific protein in humans (depending on the availability of sample materials). This is pivotal in the cases where the corresponding mouse model does not manifest the phenotype of the human disorder or when a model is not even feasible, as in case of the absence of orthologous genes.[123] Caspase 8 is a particularly striking example in these aspects. Caspase 8 disruption is lethal in mice, whereas in humans it presents itself as a lymphoproliferative and immunodeficiency syndrome. As caspase 10, a close caspase 8 paralogue, does not exist in the mouse, this divergence was inferred to arise by virtue of partial functional redundancy between the two human proteins. In addition, the phenotype of caspase 8 deficiency in humans includes a default in immune cell maturation, indicative of a non-apoptotic function of caspase 8 that may not have been uncovered otherwise.

Another remarkable example of an unexpected function unravelled by the detection of causative mutation is neutrophil elastase. While this enzyme is well known for its destructive effect on tissues at the site of inflammation, its implication in neutropenia has revealed an unforeseen role in cell fate determination.

Identification of the implicated protein and elucidation of the molecular mechanisms leading to the disorder will help to develop new therapeutic agents capable of reversing abnormal phenotypes. Strategies should be adapted depending on whether there is an overactivation or an inactivation of the implicated protease. An overactivation of a protease could be theoretically counterbalanced by any means to suppress this activation: specific inhibitor or small RNA.[124] Loss of proteolytic activity could be compensated by replacing the defective enzyme with its normal counterpart either as protein or as coding sequence or by intervention downstream of its physiological action. Many of these strategies are promising and should give rise to new efficient therapeutic agents for these disorders in the near future. They may also prove useful in pathological situations such as cancer or inflammation, in which the proteolytic systems are deregulated as secondary pathological consequences.

ACKNOWLEDGEMENTS

I would like to acknowledge Dr D Bechet for the initial idea of this manuscript. I thank Drs N Daniele, S Lupton, M Bartoli, A Bernot, and O Danos for critical reading of the manuscript and helpful suggestions. This work was supported by the Association Française contre les Myopathies.

Competing interests: none declared

REFERENCES

1 **Whitcomb DC**, Gorry MC, Preston RA, Furey W, Sossenheimer MJ, Ulrich CD, Martin SP, Gates LK Jr, Amann ST, Toskes PP, Liddle R, McGrath K, Uomo G, Post JC, Ehrlich GD. Hereditary pancreatitis is caused by a mutation in the cationic trypsinogen gene. *Nat Genet* 1996;**14**:141–5.
2 **O'Reilly DA**, Yang BM, Creighton JE, Demaine AG, Kingsnorth AN. Mutations of the cationic trypsinogen gene in hereditary and non-hereditary pancreatitis. *Digestion* 2001;**64**:54–60.
3 **Sahin-Toth M**, Toth M. Gain-of-function mutations associated with hereditary pancreatitis enhance autoactivation of human cationic trypsinogen. *Biochem Biophys Res Commun* 2000;**278**:286–9.
4 **Sahin-Toth M**. Hereditary pancreatitis-associated mutation asn(21)→ile stabilizes rat trypsinogen in vitro. *J Biol Chem* 1999;**274**:29699–704.
5 **Holzinger A**, Maier EM, Buck C, Mayerhofer PU, Kappler M, Haworth JC, Moroz SP, Hadorn HB, Sadler JE, Roscher AA. Mutations in the proenteropeptidase gene are the molecular cause of congenital enteropeptidase deficiency. *Am J Hum Genet* 2002;**70**:20–5.
6 **Mannucci PM**, Tuddenham EG. The hemophilias—from royal genes to gene therapy. *N Engl J Med* 2001;**344**:1773–9.
7 **Peyvandi F**, Duga S, Akhavan S, Mannucci PM. Rare coagulation deficiencies. *Haemophilia* 2002;**8**:308–21.
8 **Rosenberg RD**, Bauer KA. Thrombosis in inherited deficiencies of antithrombin, protein C, and protein S. *Hum Pathol* 1987;**18**:253–62.
9 **Bauer KA**. The thrombophilias: well-defined risk factors with uncertain therapeutic implications. *Ann Intern Med* 2001;**135**:367–73.
10 **Walport MJ**. Complement. First of two parts. *N Engl J Med* 2001;**344**:1058–66.
11 **Walport MJ**. Complement. Second of two parts. *N Engl J Med* 2001;**344**:1140–4.
12 **Day NK**, Geiger H, Stroud R, DeBracco M, Mancaido B, Windhorst D, Good RA. C1r deficiency: an inborn error associated with cutaneous and renal disease. *J Clin Invest* 1972;**51**:1102–8.
13 Johnson CA, Densen P, Hurford RK Jr, Colten HR, Wetsel RA Type I human complement C2 deficiency. A 28-base pair gene deletion causes skipping of exon 6 during RNA splicing. *J Biol Chem* 1992;**267**:9347–53 (Erratum in *J Biol Chem*, 1993;**268**:2268).
14 **Wetsel RA**, Kulics J, Lokki ML, Kiepiela P, Akama H, Johnson CA, Densen P, Colten HR. Type II human complement C2 deficiency. Allele-specific amino acid substitutions (Ser189→Phe; Gly444→Arg) cause impaired C2 secretion. *J Biol Chem* 1996;**271**:5824–31.
15 **Inoue N**, Saito T, Masuda R, Suzuki Y, Ohtomi M, Sakiyama H. Selective complement C1s deficiency caused by homozygous four-base deletion in the C1s gene. *Hum Genet* 1998;**103**:415–18.
16 **Stengaard-Pedersen K**, Thiel S, Gadjeva M, Moller-Kristensen M, Sorensen R, Jensen LT, Sjoholm AG, Fugger L, Jensenius JC. Inherited deficiency of mannan-binding lectin-associated serine protease 2. *N Engl J Med* 2003;**349**:554–60.
17 **Vyse TJ**, Morley BJ, Bartok I, Theodoridis EL, Davies KA, Webster AD, Walport MJ. The molecular basis of hereditary complement factor I deficiency. *J Clin Invest* 1996;**97**:925–33.
18 **Molinari F**, Rio M, Meskenaite V, Encha-Razavi F, Auge J, Bacq D, Briault S, Vekemans M, Munnich A, Attie-Bitach T, Sonderegger P, Colleaux L. Truncating neurotrypsin mutation in autosomal recessive nonsyndromic mental retardation. *Science* 2002;**298**:1779–81.
19 **Gschwend TP**, Krueger SR, Kozlov SV, Wolfer DP, Sonderegger P. Neurotrypsin, a novel multidomain serine protease expressed in the nervous system. *Mol Cell Neurosci* 1997;**9**:207–19.
20 **Scott HS**, Kudoh J, Wattenhofer M, Shibuya K, Berry A, Chrast R, Guipponi M, Wang J, Kawasaki K, Asakawa S, Minoshima S, Younus F, Mehdi SQ, Radhakrishna U, Papasavvas MP, Gehrig C, Rossier C, Korostishevsky M, Gal A, Shimizu N, Bonne-Tamir B, Antonarakis SE. Insertion of beta-satellite repeats identifies a transmembrane protease causing both congenital and childhood onset autosomal recessive deafness. *Nat Genet* 2001;**27**:59–63.
21 **Guipponi M**, Vuagniaux G, Wattenhofer M, Shibuya K, Vazquez M, Dougherty L, Scamuffa N, Guida E, Okui M, Rossier C, Hancock M, Buchet K, Reymond A, Hummler E, Marzella PL, Kudoh J, Shimizu N, Scott HS, Antonarakis SE, Rossier BC. The transmembrane serine protease (TMPRSS3) mutated in deafness DFNB8/10 activates the epithelial sodium channel (ENaC) in vitro. *Hum Mol Genet* 2002;**11**:2829–36.
22 **Couloigner V**, Fay M, Djelidi S, Farman N, Escoubet B, Runembert I, Sterkers O, Friedlander G, Ferrary E. Location and function of the epithelial Na channel in the cochlea. *Am J Physiol Renal Physiol* 2001;**280**:F214–22.

23 **Jackson RS**, Creemers JW, Ohagi S, Raffin-Sanson ML, Sanders L, Montague CT, Hutton JC, O'Rahilly S. Obesity and impaired prohormone processing associated with mutations in the human prohormone convertase 1 gene. *Nat Genet* 1997;**16**:303–6.
24 **O'Rahilly S**, Gray H, Humphreys PJ, Krook A, Polonsky KS, White A, Gibson S, Taylor K, Carr C. Brief report: impaired processing of prohormones associated with abnormalities of glucose homeostasis and adrenal function. *N Engl J Med* 1995;**333**:1386–90.
25 **Abifadel M**, Varret M, Rabes JP, Allard D, Ouguerram K, Devillers M, Cruaud C, Benjannet S, Wickham L, Erlich D, Derre A, Villeger L, Farnier M, Beucler I, Bruckert E, Chambaz J, Chanu B, Lecerf JM, Luc G, Moulin P, Weissenbach J, Prat A, Krempf M, Junien C, Seidah NG, Boileau C. Mutations in PCSK9 cause autosomal dominant hypercholesterolemia. *Nat Genet* 2003;**34**:154–6.
26 **Timms KM**, Wagner S, Samuels ME, Forbey K, Goldfine H, Jammulapati S, Skolnick MH, Hopkins PN, Hunt SC, Shattuck DM. A mutation in PCSK9 causing autosomal-dominant hypercholesterolemia in a Utah pedigree. *Hum Genet* 2004;**114**:349–53.
27 **Seidah NG**, Benjannet S, Wickham L, Marcinkiewicz J, Jasmin SB, Stifani S, Basak A, Prat A, Chretien M. The secretory proprotein convertase neural apoptosis-regulated convertase 1 (NARC-1): liver regeneration and neuronal differentiation. *Proc Natl Acad Sci USA* 2003;**100**:928–33.
28 **Maxwell KN**, Breslow JL. Adenoviral-mediated expression of Pcsk9 in mice results in a low-density lipoprotein receptor knockout phenotype. *Proc Natl Acad Sci USA* 2004;**101**:7100–5.
29 **Horwitz M**, Benson KF, Person RE, Aprikyan AG, Dale DC. Mutations in ELA2, encoding neutrophil elastase, define a 21-day biological clock in cyclic haematopoiesis. *Nat Genet* 1999;**23**:433–6.
30 **Dale DC**, Person RE, Bolyard AA, Aprikyan AG, Bos C, Bonilla MA, Boxer LA, Kannourakis G, Zeidler C, Welte K, Benson KF, Horwitz M. Mutations in the gene encoding neutrophil elastase in congenital and cyclic neutropenia. *Blood* 2000;**96**:2317–22.
31 **Horwitz M**, Benson KF, Duan Z, Person RE, Wechsler J, Williams K, Albani D, Li FQ. Role of neutrophil elastase in bone marrow failure syndromes: molecular genetic revival of the chalone hypothesis. *Curr Opin Hematol* 2003;**10**:49–54.
32 **Benson KF**, Li FQ, Person RE, Albani D, Duan Z, Wechsler J, Meade-White K, Williams K, Acland GM, Niemeyer G, Lothrop CD, Horwitz M. Mutations associated with neutropenia in dogs and humans disrupt intracellular transport of neutrophil elastase. *Nat Genet* 2003;**35**:90–6.
33 **Horwitz M**, Benson KF, Duan Z, Person RE, Wechsler J, Williams K, Albani D, Li FQ. Hereditary neutropenia: dogs explain human neutrophil elastase mutations. *Trends Mol Med* 2004;**10**:163–70.
34 **Duan Z**, Li FQ, Wechsler J, Meade-White K, Williams K, Benson KF, Horwitz M. A novel notch protein, N2N, targeted by neutrophil elastase and implicated in hereditary neutropenia. *Mol Cell Biol* 2004;**24**:58–70.
35 **Hunter MG**, Druhan LJ, Massullo PR, Avalos BR. Proteolytic cleavage of granulocyte colony-stimulating factor and its receptor by neutrophil elastase induces growth inhibition and decreased cell surface expression of the granulocyte colony-stimulating factor receptor. *Am J Hematol* 2003;**74**:149–55.
36 **Hong SE**, Shugart YY, Huang DT, Shahwan SA, Grant PE, Hourihane JO, Martin ND, Walsh CA. Autosomal recessive lissencephaly with cerebellar hypoplasia is associated with human RELN mutations. *Nat Genet* 2000;**26**:93–6.
37 **Assadi AH**, Zhang G, Beffert U, McNeil RS, Renfro AL, Niu S, Quattrocchi CC, Antalffy BA, Sheldon M, Armstrong DD, Wynshaw-Boris A, Herz J, D'Arcangelo G, Clark GD. Interaction of reelin signaling and Lis1 in brain development. *Nat Genet* 2003;**35**:270–6.
38 **Quattrocchi CC**, Wannenes F, Persico AM, Ciafre SA, D'Arcangelo G, Farace MG, Keller F. Reelin is a serine protease of the extracellular matrix. *J Biol Chem* 2002;**277**:303–9.
39 **Sleat DE**, Donnelly RJ, Lackland H, Liu CG, Sohar I, Pullarkat RK, Lobel P. Association of mutations in a lysosomal protein with classical late-infantile neuronal ceroid lipofuscinosis. *Science* 1997;**277**:1802–5.
40 **Zhou XY**, Galjart NJ, Willemsen R, Gillemans N, Galjaard H, d'Azzo A. A mutation in a mild form of galactosialidosis impairs dimerization of the protective protein and renders it unstable. *Embo J* 1991;**10**:4041–8.
41 **D'Azzo A**, Hoogeveen A, Reuser AJ, Robinson D, Galjaard H. Molecular defect in combined beta-galactosidase and neuraminidase deficiency in man. *Proc Natl Acad Sci USA* 1982;**79**:4535–9.
42 **Egeblad M**, Werb Z. New functions for the matrix metalloproteinases in cancer progression. *Nat Rev Cancer* 2002;**2**:161–74.
43 **Yong VW**, Power C, Forsyth P, Edwards DR. Metalloproteinases in biology and pathology of the nervous system. *Nat Rev Neurosci* 2001;**2**:502–11.
44 **Brinckerhoff CE**, Matrisian LM. Matrix metalloproteinases: a tail of a frog that became a prince. *Nat Rev Mol Cell Biol* 2002;**3**:207–14.
45 **Martignetti JA**, Aqeel AA, Sewairi WA, Boumah CE, Kambouris M, Mayouf SA, Sheth KV, Eid WA, Dowling O, Harris J, Glucksman MJ, Bahabri S, Meyer BF, Desnick RJ. Mutation of the matrix metalloproteinase 2 gene (MMP2) causes a multicentric osteolysis and arthritis syndrome. *Nat Genet* 2001;**28**:261–5.
46 **Okada Y**, Morodomi T, Enghild JJ, Suzuki K, Yasui A, Nakanishi I, Salvesen G, Nagase H. Matrix metalloproteinase 2 from human rheumatoid synovial fibroblasts. Purification and activation of the precursor and enzymic properties. *Eur J Biochem* 1990;**194**:721–30.
47 **Colige A**, Sieron AL, Li SW, Schwarze U, Petty E, Wertelecki W, Wilcox W, Krakow D, Cohn DH, Reardon W, Byers PH, Lapiere CM, Prockop DJ, Nusgens BV. Human Ehlers-Danlos syndrome type VII C and bovine dermatosparaxis are caused by mutations in the procollagen I N-proteinase gene. *Am J Hum Genet* 1999;**65**:308–17.
48 **Lapiere CM**, Lenaers A, Kohn LD. Procollagen peptidase: an enzyme excising the coordination peptides of procollagen. *Proc Natl Acad Sci USA* 1971;**68**:3054–8.
49 **Levy GG**, Nichols WC, Lian EC, Foroud T, McClintick JN, McGee BM, Yang AY, Siemieniak DR, Stark KR, Gruppo R, Sarode R, Shurin SB, Chandrasekaran V, Stabler SP, Sabio H, Bouhassira EE, Upshaw JD Jr, Ginsburg D, Tsai HM. Mutations in a member of the ADAMTS gene family cause thrombotic thrombocytopenic purpura. *Nature* 2001;**413**:488–94.
50 **Verweij CL**. Biosynthesis of human von Willebrand factor. *Haemostasis* 1988;**18**:224–45.
51 **Turner AJ**, Isaac RE, Coates D. The neprilysin (NEP) family of zinc metalloendopeptidases: genomics and function. *Bioessays* 2001;**23**:261–9.
52 **Hofstra RM**, Valdenaire O, Arch E, Osinga J, Kroes H, Loffler BM, Hamosh A, Meijers C, Buys CH. A loss-of-function mutation in the endothelin-converting enzyme 1 (ECE-1) associated with Hirschsprung disease, cardiac defects, and autonomic dysfunction. *Am J Hum Genet* 1999;**64**:304–8.
53 **Yanagisawa H**, Yanagisawa M, Kapur RP, Richardson JA, Williams SC, Clouthier DE, de Wit D, Emoto N, Hammer RE. Dual genetic pathways of endothelin-mediated intercellular signaling revealed by targeted disruption of endothelin converting enzyme-1 gene. *Development* 1998;**125**:825–36.
54 **Dupin E**, Real C, Ledouarin N. The neural crest stem cells: control of neural crest cell fate and plasticity by endothelin-3. *An Acad Bras Cienc* 2001;**73**:533–45.
55 **Iwashita T**, Kruger GM, Pardal R, Kiel MJ, Morrison SJ. Hirschsprung disease is linked to defects in neural crest stem cell function. *Science* 2003;**301**:972–6.
56 **Francis F**, Hennig S, Korn B, Reinhardt R, de Jong P, Poustka A, Lehrach H, Rowe PSN, Goulding JN, Summerfield T, Mountford R, Read AP, Popowska E, Pronicka E, Davies KE, O'Riordan JLH, Econs MJ, Nesbitt T, Drezner MK, Oudet C, Pannetier S, Hanauer A, Strom TM, Meindl A, Lorenz B, Cagnoli B, Mohnike KL, Murken J, Meitinger T. A gene (PEX) with homologies to endopeptidases is mutated in patients with X-linked hypophosphatemic rickets. The HYP Consortium. *Nat Genet* 1995;**11**:130–6.
57 **Drezner MK**. PHEX gene and hypophosphatemia. *Kidney Int* 2000;**57**:9–18.
58 **Xiao ZS**, Crenshaw M, Guo R, Nesbitt T, Drezner MK, Quarles LD. Intrinsic mineralization defect in Hyp mouse osteoblasts. *Am J Physiol* 1998;**275**:e700–8.
59 **Agarwal AK**, Fryns JP, Auchus RJ, Garg A. Zinc metalloproteinase, ZMPSTE24, is mutated in mandibuloacral dysplasia. *Hum Mol Genet* 2003;**12**:1995–2001.
60 **Pendas AM**, Zhou Z, Cadinanos J, Freije JM, Wang J, Hultenby K, Astudillo A, Wernerson A, Rodriguez F, Tryggvason K, Lopez-Otin C. Defective prelamin A processing and muscular and adipocyte alterations in Zmpste24 metalloproteinase-deficient mice. *Nat Genet* 2002;**31**:94–9.
61 **Casari G**, De Fusco M, Ciarmatori S, Zeviani M, Mora M, Fernandez P, De Michele G, Filla A, Cocozza S, Marconi R, Durr A, Fontaine B, Ballabio A. Spastic paraplegia and OXPHOS impairment caused by mutations in paraplegin, a nuclear-encoded mitochondrial metalloprotease. *Cell* 1998;**93**:973–83.
62 **Arlt H**, Steglich G, Perryman R, Guiard B, Neupert W, Langer T. The formation of respiratory chain complexes in mitochondria is under the proteolytic control of the m-AAA protease. *Embo J* 1998;**17**:4837–47.
63 **Ferreirinha F**, Quattrini A, Pirozzi M, Valsecchi V, Dina G, Broccoli V, Auricchio A, Piemonte F, Tozzi G, Gaeta L, Casari G, Ballabio A, Rugarli EI. Axonal degeneration in paraplegin-deficient mice is associated with abnormal mitochondria and impairment of axonal transport. *J Clin Invest* 2004;**113**:231–42.
64 **Tandon A**, Fraser P. The presenilins. *Genome Biol* 2002;**3**:3014.
65 **Kimberly WT**, Wolfe MS. Identity and function of gamma-secretase. *J Neurosci Res* 2003;**74**:353–60.
66 **Medina M**, Dotti CG. RIPped out by presenilin-dependent gamma-secretase. *Cell Signal* 2003;**15**:829–41.
67 **Brown MS**, Ye J, Rawson RB, Goldstein JL. Regulated intramembrane proteolysis: a control mechanism conserved from bacteria to humans. *Cell* 2000;**100**:391–8.
68 **Takeda K**, Araki W, Tabira T. Enhanced generation of intracellular Abeta42 amyloid peptide by mutation of presenilins PS1 and PS2. *Eur J Neurosci* 2004;**19**:258–264.
69 **Berdowska I**. Cysteine proteases as disease markers. *Clin Chim Acta* 2004;**342**:41–69.
70 **Goulet B**, Baruch A, Moon NS, Poirier M, Sansregret LL, Erickson A, Bogyo M, Nepveu A. A cathepsin L isoform that is devoid of a signal peptide localizes to the nucleus in S phase and processes the CDP/Cux transcription factor. *Mol Cell* 2004;**14**:207–19.
71 **Everts V**, Hou WS, Rialland X, Tigchelaar W, Saftig P, Bromme D, Gelb BD, Beertsen W. Pycnodysostosis, a lysosomal disease caused by cathepsin K deficiency. *Science* 1996;**273**:1236–8.
72 **Everts V**, Hou WS, Rialland X, Tigchelaar W, Saftig P, Bromme D, Gelb BD, Beertsen W. Cathepsin K deficiency in pycnodysostosis results in accumulation of non-digested phagocytosed collagen in fibroblasts. *Calcif Tissue Int* 2003;**73**:380–6.
73 **Toomes C**, James J, Wood AJ, Wu CL, McCormick D, Lench N, Hewitt C, Moynihan L, Roberts E, Woods CG, Markham A, Wong M, Widmer R, Ghaffar KA, Pemberton M, Hussein IR, Temtamy SA, Davies R, Read AP, Sloan P, Dixon MJ, Thakker NS. Loss-of-function mutations in the cathepsin C gene result in periodontal disease and palmoplantar keratosis. *Nat Genet* 1999;**23**:421–4.
74 **Hart TC**, Hart PS, Michalec MD, Zhang Y, Firatli E, Van Dyke TE, Stabholz A, Zlotogorski A, Shapira L, Soskolne WA. Haim-Munk syndrome and

Papillon-Lefevre syndrome are allelic mutations in cathepsin C. *J Med Genet* 2000;**37**:88–94.
75 **Adkison AM**, Raptis SZ, Kelley DG, Pham CT. Dipeptidyl peptidase I activates neutrophil-derived serine proteases and regulates the development of acute experimental arthritis. *J Clin Invest* 2002;**109**:363–71.
76 **Goll DE**, Thompson VF, Li H, Wei W, Cong J. The calpain system. *Physiol Rev* 2003;**83**:731–801.
77 **Huang Y**, Wang KK. The calpain family and human disease. *Trends Mol Med* 2001;**7**:355–62.
78 **Yoshikawa Y**, Mukai H, Hino F, Asada K, Kato I. Isolation of two novel genes, down-regulated in gastric cancer. *Jpn J Cancer Res* 2000;**91**:459–63.
79 **Cox NJ**, Hayes MG, Roe CA, Tsuchiya T, Bell GI. Linkage of calpain 10 to type 2 diabetes: the biological rationale. *Diabetes* 2004;**53**(suppl 1):S19–25.
80 **Richard I**, Broux O, Allamand V, Fougerousse F, Chiannilkulchai N, Bourg N, Brenguier L, Devaud C, Pasturaud P, Roudaut C, Hillaire D, Passos-Bueno M-R, Zatz M, Tischfield JA, Fardeau M, Jackson CE, Cohen D, Beckmann JS. Mutations in the proteolytic enzyme, calpain 3, cause limb-girdle muscular dystrophy type 2A. *Cell* 1995;**81**:27–40.
81 **Fardeau M**, Eymard B, Mignard C, Tome FM, Richard I, Beckmann JS. Chromosome 15-linked limb-girdle muscular dystrophy: clinical phenotypes in Reunion Island and French metropolitan communities. *Neuromuscul Disord* 1996;**6**:447–53.
82 **Taveau M**, Bourg N, Sillon G, Roudaut C, Bartoli M, Richard I. Calpain 3 is activated through autolysis within the active site and lyses sarcomeric and sarcolemmal components. *Mol Cell Biol* 2003;**23**:9127–35.
83 **Baghdiguian S**, Martin M, Richard I, Pons F, Astier C, Bourg N, Hay RT, Chemaly R, Halaby G, Loiselet J, Anderson LV, Lopez de Munain A, Fardeau M, Mangeat P, Beckmann JS, Lefranc G. Calpain 3 deficiency is associated with myonuclear apoptosis and profound perturbation of the IkappaB alpha/NF-kappaB pathway in limb-girdle muscular dystrophy type 2A. *Nat Med* 1999;**5**:503–11.
84 **Earnshaw WC**, Martins LM, Kaufmann SH. Mammalian caspases: structure, activation, substrates, and functions during apoptosis. *Annu Rev Biochem* 1999;**68**:383–424.
85 **Rossi D**, Gaidano G. Messengers of cell death: apoptotic signaling in health and disease. *Haematologica* 2003;**88**:212–18.
86 **Saikumar P**, Dong Z, Mikhailov V, Denton M, Weinberg JM, Venkatachalam MA. Apoptosis: definition, mechanisms, and relevance to disease. *Am J Med* 1999;**107**:489–506.
87 **Wang J**, Zheng L, Lobito A, Chan FK, Dale J, Sneller M, Yao X, Puck JM, Straus SE, Lenardo MJ. Inherited human Caspase 10 mutations underlie defective lymphocyte and dendritic cell apoptosis in autoimmune lymphoproliferative syndrome type II. *Cell* 1999;**98**:47–58.
88 **Chun HJ**, Zheng L, Ahmad M, Wang J, Speirs CK, Siegel RM, Dale JK, Puck J, Davis J, Hall CG, Skoda-Smith S, Atkinson TP, Straus SE, Lenardo MJ. Pleiotropic defects in lymphocyte activation caused by caspase-8 mutations lead to human immunodeficiency. *Nature* 2002;**419**:395–9.
89 **Pickart CM**, Cohen RE. Proteasomes and their kin: proteases in the machine age. *Nat Rev Mol Cell Biol* 2004;**5**:177–87.
90 **Wilkinson KD**. Regulation of ubiquitin-dependent processes by deubiquitinating enzymes. *Faseb J* 1997;**11**:1245–56.
91 **Leroy E**, Boyer R, Auburger G, Leube B, Ulm G, Mezey E, Harta G, Brownstein MJ, Jonnalagada S, Chernova T, Dehejia A, Lavedan C, Gasser T, Steinbach PJ, Wilkinson KD, Polymeropoulos MH. The ubiquitin pathway in Parkinson's disease. *Nature* 1998;**395**:451–2.
92 **Clayton DF**, George JM. The synucleins: a family of proteins involved in synaptic function, plasticity, neurodegeneration and disease. *Trends Neurosci* 1998;**21**:249–54.
93 **Liu Y**, Fallon L, Lashuel HA, Liu Z, Lansbury PT Jr. The UCH-L1 gene encodes two opposing enzymatic activities that affect alpha-synuclein degradation and Parkinson's disease susceptibility. *Cell* 2002;**111**:209–18.
94 **Maraganore DM**, Farrer MJ, Lesnick TG, de Andrade M, Bower JH, Hernandez D, Hardy JA, Rocca WA. Case-control study of the ubiquitin carboxy-terminal hydrolase L1 gene in Parkinson's disease. *Neurology* 1999;**53**:1858–60.
95 **Bignell GR**, Warren W, Seal S, Takahashi M, Rapley E, Barfoot R, Green H, Brown C, Biggs PJ, Lakhani SR, Jones C, Hansen J, Blair E, Hofmann B, Siebert R, Turner G, Evans DG, Schrander-Stumpel C, Beemer FA, van Den Ouweland A, Halley D, Delpech B, Cleveland MG, Leigh I, Leisti J, Rasmussen S. Identification of the familial cylindromatosis tumour-suppressor gene. *Nat Genet* 2000;**25**:160–5.
96 **Zhang XJ**, Liang YH, He PP, Yang S, Wang HY, Chen JJ, Yuan WT, Xu SJ, Cui Y, Huang W. Identification of the cylindromatosis tumor-suppressor gene responsible for multiple familial trichoepithelioma. *J Invest Dermatol* 2004;**122**:658–64.
97 **Brummelkamp TR**, Nijman SM, Dirac AM, Bernards R. Loss of the cylindromatosis tumour suppressor inhibits apoptosis by activating NF-kappaB. *Nature* 2003;**424**:797–801.
98 **Kovalenko A**, Chable-Bessia C, Cantarella G, Israel A, Wallach D, Courtois G. The tumour suppressor CYLD negatively regulates NF-kappaB signalling by deubiquitination. *Nature* 2003;**424**:801–5.
99 **Trompouki E**, Hatzivassiliou E, Tsichritzis T, Farmer H, Ashworth A, Mosialos G. CYLD is a deubiquitinating enzyme that negatively regulates NF-kappaB activation by TNFR family members. *Nature* 2003;**424**:793–6.
100 **Deng L**, Wang C, Spencer E, Yang L, Braun A, You J, Slaughter C, Pickart C, Chen ZJ. Activation of the IkappaB kinase complex by TRAF6 requires a dimeric ubiquitin-conjugating enzyme complex and a unique polyubiquitin chain. *Cell* 2000;**103**:351–61.
101 **Sun C**, Skaletsky H, Birren B, Devon K, Tang Z, Silber S, Oates R, Page DC. An azoospermic man with a de novo point mutation in the Y-chromosomal gene USP9Y. *Nat Genet* 1999;**23**:429–32.
102 **Jones MH**, Furlong RA, Burkin H, Chalmers IJ, Brown GM, Khwaja O, Affara NA. The Drosophila developmental gene fat facets has a human homologue in Xp11.4 which escapes X-inactivation and has related sequences on Yq11.2. *Hum Mol Genet* 1996:1695–701.
103 **Fischer-Vize JA**, Rubin GM, Lehmann R. The fat facets gene is required for Drosophila eye and embryo development. *Development* 1992;**116**:985–1000.
104 **Fay WP**, Shapiro AD, Shih JL, Schleef RR, Ginsburg D. Brief report: complete deficiency of plasminogen-activator inhibitor type 1 due to a frame-shift mutation. *N Engl J Med* 1992;**327**:1729–33.
105 **Weber BH**, Vogt G, Pruett RC, Stohr H, Felbor U. Mutations in the tissue inhibitor of metalloproteinases-3 (TIMP3) in patients with Sorsby's fundus dystrophy. *Nat Genet* 1994;**8**:352–6.
106 **Pennacchio LA**, Lehesjoki AE, Stone NE, Willour VL, Virtaneva K, Miao J, D'Amato E, Ramirez L, Faham M, Koskiniemi M, Warrington JA, Norio R, de la Chapelle A, Cox DR, Myers RM. Mutations in the gene encoding cystatin B in progressive myoclonus epilepsy (EPM1). *Science* 1996;**271**:1731–4.
107 **Davis RL**, Shrimpton AE, Holohan PD, Bradshaw C, Feiglin D, Collins GH, Sonderegger P, Kinter J, Becker LM, Lacbawan F, Krasnewich D, Muenke M, Lawrence DA, Yerby MS, Shaw CM, Gooptu B, Elliott PR, Finch JT, Carrell RW, Lomas DA. Familial dementia caused by polymerization of mutant neuroserpin. *Nature* 1999;**401**:376–9.
108 **Witt H**, Luck W, Hennies HC, Classen M, Kage A, Lass U, Landt O, Becker M. Mutations in the gene encoding the serine protease inhibitor, Kazal type 1 are associated with chronic pancreatitis. *Nat Genet* 2000;**25**:213–16.
109 **Pickart CM**. Mechanisms underlying ubiquitination. *Annu Rev Biochem* 2001;**70**:503–33.
110 **Kishino T**, Lalande M, Wagstaff J. UBE3A/E6-AP mutations cause Angelman syndrome. *Nat Genet* 1997;**15**:70–3.
111 **Consortium**. TF-GA. An autoimmune disease, APECED, caused by mutations in a novel gene featuring two PHD-type zinc-finger domains. The Finnish-German APECED Consortium. Autoimmune Polyendocrinopathy-Candidiasis-Ectodermal Dystrophy. *Nat Genet* 1997;**17**:399–403.
112 **Matsuura T**, Sutcliffe JS, Fang P, Galjaard RJ, Jiang YH, Benton CS, Rommens JM, Beaudet AL. De novo truncating mutations in E6-AP ubiquitin-protein ligase gene (UBE3A) in Angelman syndrome. *Nat Genet* 1997;**15**:74–7.
113 **Nagamine K**, Peterson P, Scott HS, Kudoh J, Minoshima S, Heino M, Krohn KJ, Lalioti MD, Mullis PE, Antonarakis SE, Kawasaki K, Asakawa S, Ito F, Shimizu N. Positional cloning of the APECED gene. *Nat Genet* 1997;**17**:393–8.
114 **Kitada T**, Asakawa S, Hattori N, Matsumine H, Yamamura Y, Minoshima S, Yokochi M, Mizuno Y, Shimizu N. Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. *Nature* 1998;**392**:605–8.
115 **Meriluoto T**, Halonen M, Pelto-Huikko M, Kangas H, Korhonen J, Kolmer M, Ulmanen I, Eskelin P. The autoimmune regulator: a key toward understanding the molecular pathogenesis of autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy. *Keio J Med* 2001;**50**:225–39.
116 **Frosk P**, Weiler T, Nylen E, Sudha T, Greenberg CR, Morgan K, Fujiwara TM, Wrogemann. Limb-girdle muscular dystrophy type 2H associated with mutation in TRIM32, a putative E3-ubiquitin-ligase gene. *Am J Hum Genet* 2002;**70**:663–72.
117 **Hampton RY**. ER-associated degradation in protein quality control and cellular regulation. *Curr Opin Cell Biol* 2002;**14**:476–82.
118 **Chan EM**, Young EJ, Ianzano L, Munteanu I, Zhao X, Christopoulos CC, Avanzini G, Elia M, Ackerley CA, Jovic NJ, Bohlega S, Andermann E, Rouleau GA, Delgado-Escueta AV, Minassian BA, Scherer SW. Mutations in NHLRC1 cause progressive myoclonus epilepsy. *Nat Genet* 2003;**35**:125–7.
119 **Meetei AR**, de Winter JP, Medhurst AL, Wallisch M, Waisfisz Q, van de Vrugt HJ, Oostra AB, Yan Z, Ling C, Bishop CE, Hoatlin ME, Joenje H, Wang W. A novel ubiquitin ligase is deficient in Fanconi anemia. *Nat Genet* 2003;**35**:165–70.
120 **Tsai YC**, Fishman PS, Thakor NV, Oyler GA. Parkin facilitates the elimination of expanded polyglutamine proteins and leads to preservation of proteasome function. *J Biol Chem* 2003;**278**:22044–55.
121 **Bossy-Wetzel E**, Schwarzenbacher R, Lipton SA. Molecular pathways to neurodegeneration. *Nat Med* 2004;**10**(suppl):S2–9.
122 **Grune T**, Jung T, Merker K, Davies KJ. Decreased proteolysis caused by protein aggregates, inclusion bodies, plaques, lipofuscin, ceroid, and ''aggresomes'' during oxidative stress, aging, and disease. *Int J Biochem Cell Biol* 2004;**36**:2519–30.
123 **Puente XS**, Sanchez LM, Overall CM, Lopez-Otin C. Human and mouse proteases: a comparative genomic approach. *Nat Rev Genet* 2003;**4**:544–58.
124 **Caplen NJ**. Gene therapy progress and prospects. Downregulating gene expression: the impact of RNA interference, *Gene Ther* 2004;**11**:1241–8.